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Susceptibility of cypress seedlings to the eriophyoid mite *Trisetacus juniperinus*

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Abstract. In Italian nurseries and young groves of evergreen cypress (*Cupressus sempervirens* L.), the eriophyoid mite *Trisetacus juniperinus* (Nal.) is considered a very serious pest. A rating system of damage symptoms was developed to investigate the susceptibility of different cypress seedling families to the mite. Based on this system, the seedlings were evaluated for three years in the nursery and in two field locations after transplanting. Data obtained in the nursery allowed the cypress families to be allocated to at least two different levels of susceptibility. These levels of susceptibility were generally also maintained in the field. However, the environmental conditions of the two transplanting localities significantly affected the susceptibility of each family. In all families, scores for each of the damage categories were strongly correlated positively to each other and negatively with the average increment in the height of plant over the duration of the field experiment. Assessment of the intensity of the symptoms peculiar to damage category A (buds enlarged, deformed, russet and/or branch apex folded) was sufficient to give the same susceptibility evaluation as if data for all damage categories were used. The evaluation of susceptibility on the basis of injury pattern may return very useful information for selection and certification of families of known susceptibility to eriophyoid mites.

Introduction

In Italy, the evergreen cypress (*Cupressus sempervirens* L.) is widely cultivated for its ornamental value, suitability for arid soils, and quality of its wood. In recent years, however, the severity of injuries caused by the eriophyoid mite *Trisetacus juniperinus* (Nal.) has become increasingly serious in nurseries and young groves, and the mite is considered one of the key pests of young cultivated plants (Castagnoli and Simoni 1998) along with the fungal disease cypress canker, *Seiridium cardinale* (Wag.) Sutton & Gibson (Panconesi and Raddi 1998). The mite lives hidden in apical and sub-apical buds where populations develop continuously throughout the year (Castagnoli and Simoni 2000). In natural stands, the infestations of *T. juniperinus* usually remain at low levels and mature plants are generally able to withstand localized injuries (Castagnoli 1996). On the contrary, in artificial conditions the trees are often heavily infested, especially when their growth is forced. In this case the buds swell, at times either wither and degenerate or proliferate abnormally; the plants often have deformed apical branches, known as witches' brooms, and cones that produce few seeds. When the plants lose their aesthetic value, economic loss can be considerable. Some pesticides can reduce the eriophyoid populations (Nuzzaci and Monaco 1977), but it is very hard to achieve effective prevention of injuries only using chemical treatments, due to the complexity of host-eriophyoid interactions (Westhphal and Manson 1996). Furthermore, the microscopic size and sheltered habitats of these mites make it difficult to study their behaviour and almost impossible to monitor their populations by direct count. To account for these problems, a rating system has been developed for the cypress system where damaged plant parts are assigned to categories based on a visual appraisal of the symptoms (Castagnoli and Simoni 1998), similar to those used for some *Citrus* eriophyoid pests (Royalty and Perring 1996). Our long term observations (Castagnoli and Simoni (1998, 2000)), as well as the study of Guido et al. (1995), suggest that different levels of susceptibility to mites are exhibited by different cypress individuals and injury patterns could be used as a measure of this susceptibility.

On the basis of observed and categorised damages, the principal aims of this study were to investigate (a) if families of cypress seedlings, also under consideration for the resistance to canker, evidenced different injury severity when subjected to the same risk of natural eriophyoid infestations, (b) if the differences recorded in the injury pattern were affected by the transplant locality and/or the time of plant growth, and (c) if plant height was correlated to observed injuries.

Material and Methods

Experimental procedures

For a first short period (between September, 1996 and February, 1997) the research was conducted in a nursery located at Antella (near Florence, Italy), in an area where the evergreen cypress is widespread and *T. juniperinus* endemic. The nursery usually houses some thousands of cypress seedlings of different families, selected for canker resistance by IPAF-CNR (Raddi and Panconesi 1998). The families were obtained by self-crossing or crossing with a single heterologous pollen. Both the male and female parent trees of each family have been previously tested for the degree of susceptibility to canker (Raddi and Panconesi 1998). For this study, two-year-old seedlings were chosen belonging to 15 different families (10–22 plants per family) and planted in pots. As all seedlings had been subjected to identical conditions for the same time interval, they ran the same risk of becoming infested with the eriophyoid mites from surrounding infested plants. The seedlings of each family were randomly assigned to two groups to be successively transplanted in two different localities. Three observations were made in the nursery.

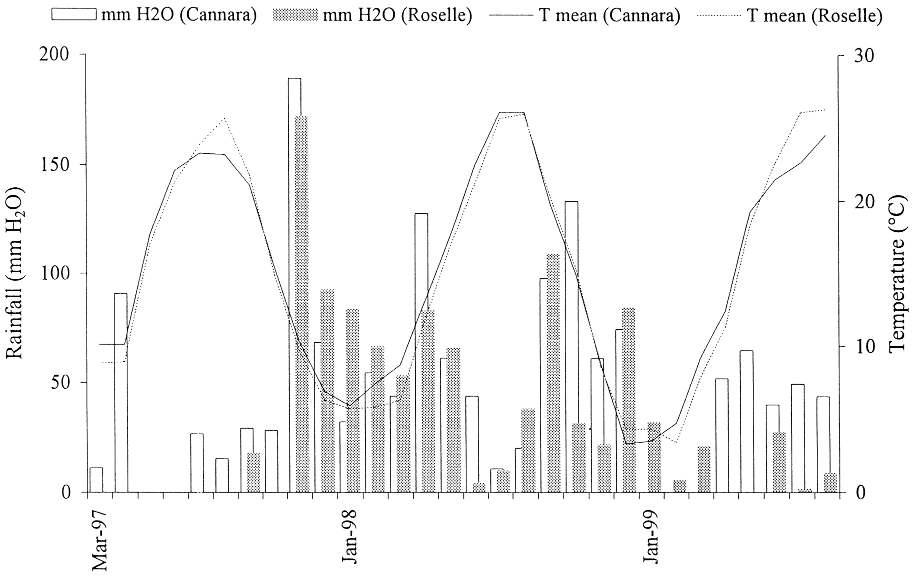

Figure 1. Monthly rainfall and average temperature registered in Cannara and Roselle fields

In March, 1997 the seedlings were transplanted in experimental plots of two commercial farms producing cypress and other plants for ornamental or reforestation use. The first was located in the hinterland, 150 metres above sea-level, at Cannara (Perugia) (42.99° lat and 12.58° long), the second at Roselle (Grosseto) (42.81° lat and 11.14° long), near the Tirrenian coast. A resume of the temperature and rainfall data of the two fields is shown in Figure 1. In each field, 6 observations (one every four months) were made beginning at the end of 1997, which allowed the plants sufficient time to overcome the transplant stress.

All observations consisted on a visual check of the whole range of damage symptoms of each plant. The symptoms were assigned to four categories (A,B,C, and D) as shown in Table 1, which were generated by combining and simplifying those already proposed by Castagnoli and Simoni (1998) and which represent possible steps in the development of the injury pattern. The severity of each symptom was graduated from 0 (absence) to 4 (the highest intensity) and plants often exhibited more than one category of damage. Both in nursery and in field, a preliminary survey confirmed a constant presence of eriophyoids when symptoms A, B (with the exception of buds completely dried) and D were found. On the contrary, when the symptom C occurred, the mites had already left the damaged and dried part of plant.

In the field the height of each plant was also recorded from ground level to the tip of the apical meristem.

Table 1. Rating systems of symptoms caused by *Trisetacus juniperinus* on *Cupressus sempervirens*

Old classification of damage categories(Castagnoli and Simoni 1998)			
a	b	c*	d
buds slightly enlarged, turgid, sometimes with light colour changes	buds enlarged, russet in the center and/or partially dried	cones deformed and producing few seeds	buds completely dried, cones dried
e	f	g	h
branch apex deformed, folded, filamentous or enlarged	branch apex dried	large areas dried on branches and stems	irregular proliferations of axillary buds, blastomania, witches' brooms, partial or total shooting
Classification of damage categories adopted in this study			
A	B	C	D
buds enlarged, deformed, russet and/or branch apex folded (=a + b partim + e)	buds more or less dried (= b partim + d partim)	brachyblasts and/or part of branches dried (= f + g)	irregular proliferations of axillary buds, blastomania, witches' brooms (= h)

	Symptom intensity scale adopted in this study				
	0	1	2	3	4
symptom A, B, C (n. records / plant)	absent	1 — 4	5 — 15	16 — 30	> 30
symptom D (% of plant damaged)	absent	< 15 %	16 — 30 %	30 — 50 %	> 50 %

*this symptom is not considered in this paper because the young plants do not produce cones

Analysis of data

Nursery

For each individual tree the raw data on injuries categorized as indicated in Table 1 and recorded in the three samples in the nursery, were pooled because they were recorded across a very short time interval. Pooled and untransformed data were analysed by means of a multivariate analysis of variance (MANOVA) to test if there were any differences between the plants that were assigned to the two different localities. Principal component analysis (PCA), followed by a cluster analysis, was used to test if the 15 families could be assigned at least to two initial susceptibility groups. All statistics were calculated on damage A, B, and D because damage C was very sporadically observed in nursery. All statistics were performed using SPSS Inc. (1999).

Field

A general linear model (GLM) analysis has been applied to all four kinds of damage, considering as explanatory variables the sampling time, the locality and the susceptibility group, as indicated by the cluster analysis performed on the nursery data. Correlation between damage categories was evaluated using Pearson's correlation coefficient. For each locality, a PCA analysis was done, similar to the nursery data, and the factor scores used to find homogeneous groups of cypress families by means of a cluster analysis. The significance of differences between the groups of the families was tested using Scheffè's simultaneous intervals. Then the same procedure was also performed considering only the damage A, the early and most common symptom of *T. juniperinus* infestation. The correlation between the increment in the plant height registered at the end of experiment, *i.e.* the change in height over the duration of the experiment, and damage factor scores was also evaluated by a linear regression. As for the nursery data, statistics were performed using SPSS Inc. (1999).

Results

Nursery

All seedlings showed symptoms of eriophyoid infestation. Damage category A represented 57% of the total injuries recorded, ranging from an average intensity per plant of 1.44 to 3.53 according to family, followed by damage category B (34%) which ranged from 0.62 to 2.02. Damage category D occurred sporadically and only one family (35) reached an average of intensity higher than 1 (Figure 2). On the basis of these damage scores, the MANOVA showed no significant differences (P = 0.05) between the two groups of plants of each family that were assigned to either Cannara or Roselle fields, and all plants of each family were therefore treated as a homogeneous group. The PCA applied to these 15 families showed only one domi-

damages A	damages B	damages D	family
1.83	1.37	0.03	51
1.91	1.33	0.15	55
1.80	1.37	0.09	4
2.14	1.31	0.33	13
2.53	1.16	0.20	21
1.98	1.83	0.25	7
2.51	1.20	0.35	28
2.24	1.51	0.47	47
1.44	1.29	0.09	6
1.64	0.62	0.23	32
2.81	2.02	0.42	18
3.46	1.36	0.49	34
3.39	1.28	0.47	36
2.95	1.95	0.82	30
3.53	1.80	1.47	35

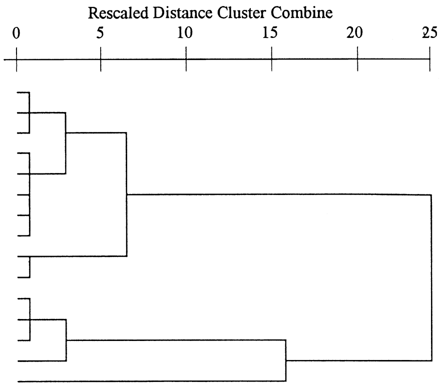

Figure 2. Nursery. Average of damages of each family, cluster composition and dendrogram based on UPGMA cluster analysis from data registered for each family. Distant measures are based on similarity/dissimilarity coefficients.

Table 2. Results of the principal component analysis applied to the damages registered in nursery and field

	Component	Eigenvalues	% of Variance	Cumulative %
nursery	1	2.09	69.61	69.61
	2	0.66	22.11	91.71
	3	0.25	8.29	100.00
fields	1	3.41	85.29	85.29
	2	0.32	8.11	93.40
	3	0.24	5.94	99.34
	4	0.03	0.66	100.00

nant factor, which explained about 70% of total variability and which was quite uniformly correlated to the three categories of damage (A,B, and D) considered (Table 2). On the basis of this factor, it was possible to obtain the dendrogram shown in Figure 2, which allowed a first classification step. The plant families were clearly divided in two groups of susceptibility: the first included the 10 less injured (from family 51 to 32 in Figure 2), while the second the remaining 5 most injured.

Field

When the plants were located in the field, all damage categories were present (Figure 3) in all samples. In Cannara, the presence of both damage categories A and B was 34% and 31% of the whole injuries, respectively, followed by 21% of C and 14% of D, while in Roselle the distribution of the same damages was 45%, 29%, 12%, and 14%.

A highly significant difference was found between the two susceptibility groups already defined from the nursery data (Figure 2), as well as between the locality and among the plant families. Sampling time explained the smallest proportion of

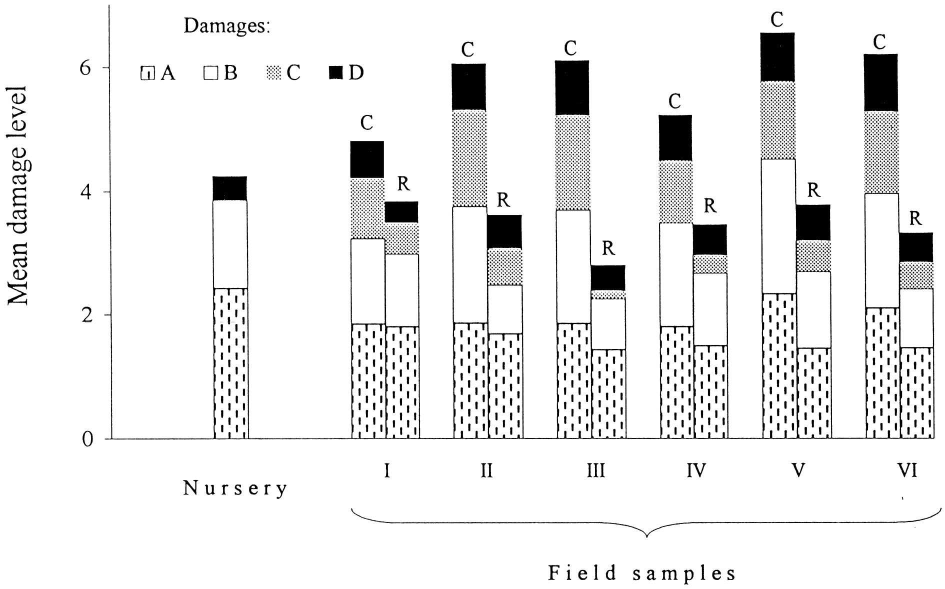

Figure 3. Average levels of damages registered in nursery and for each sample (I – VI) in two fields (C = Cannara, R = Roselle). Damage classes A-D are explained in Table 1

the total variability of the model and omitting time from the GLM analysis did not change the results of the analysis (Table 3). Evaluating the variance components in the model, it was evident for all damages that the dominant variability was always due to the two initial groups of susceptibility, followed by that of the localities, especially for damages B and C. Also the variability due to the interaction group × family was quite high and this implies that, within the same group, families showed significant differences in damage degree, whereas the interaction group×locality was significant only for damage C and D.

On the whole the damage manifestation was more intense in Cannara than in Roselle (Figure 3), where the global damage index per plant (GDI, i.e. the sum of means of four damages per plant recorded in the families) was 5.80 and 3.48, respectively.

The classification performed for each locality is shown in Figure 4. In both localities the main split showed two groups different from those already defined in the nursery data and used to separate the families in the two initial groups of susceptibility. In fact the most damaged group changes from five to three or two families in Cannara and Roselle stands, respectively. In each locality it was possible to evidence and graduate up to five homogeneous and significantly different subsets of plant families (Figure 4, Table 4). The five subsets did not show the same damage levels: the GDI ranged from 0.58 and 2.17 (subset I, the less damaged) to 7.38 and 11. 39 (subset V, the most damaged), in Roselle and in Cannara, respectively.

The PCA on data obtained from the two field localities indicated that about 85% of the variability could be explained by the first factor which was a function of the

Table 3. Analysis of variance of field damages (GLM procedure) in relation to locality, initial susceptibility group and family

		Multivariate Tests			
Effect	Wilks' Lambda	F	Hypothesis df	Error df	Sig.
locality	0.431	52.84	4	160	0.000
group	0.343	76.54	4	160	0.000
locality × group	0.817	8.97	4	160	0.000
group × family	0.106	9.38	52	621.79	0.000
		Tests of Between-Subjects Effects			
Source	Dependent Variable	Type III Sum of Squares	df	F	Sig.
locality	A	7.01	1	46.85	0.000
	B	26.54	1	107.80	0.000
	C	38.50	1	209.29	0.000
	D	5.33	1	41.18	0.000
group	A	20.48	1	136.90	0.000
	B	32.37	1	131.50	0.000
	C	26.53	1	144.26	0.000
	D	32.61	1	251.82	0.000
locality × group	A	3.0E-4	1	0.00	0.964
	B	0.59	1	2.39	0.124
	C	5.16	1	28.07	0.000
	D	1.26	1	9.74	0.002
group × family	A	26.19	13	13.47	0.000
	B	36.47	13	11.39	0.000
	C	23.39	13	9.78	0.000
	D	44.20	13	26.26	0.000

damage scores (Table 2), similar to the results for the nursery. Furthermore, the correlation coefficients between damage categories ranged between 0.248 and 0.627 and all were highly significant ($P > 0.0001$). When only damage category A was considered for each locality, the same homogeneous subsets of cypress families were obtained (Figure 5).

At the Roselle stand, the plants of each family were significantly taller than at the Cannara stand, as confirmed by a t-test (P=0.05). A negative relationship was found between damage and height increase, *i.e.* more damage resulted in a smaller increase in height. The relationship was stronger for Cannara than for Roselle (Figure 6).

Cannara

Damages A	B	C	D	Subset	GDI	Family
1.80	1.33	0.78	0.41	II	4.28	7
2.10	1.43	0.63	0.08			21
2.30	2.22	1.72	0.70	III	9.94	34
1.76	2.24	1.87	0.93			47
2.07	1.78	1.00	0.72			13
2.31	1.83	1.11	0.25			28
1.80	2.13	1.46	0.65			51
1.64	1.95	1.40	1.05			55
1.92	2.11	1.69	0.31			36
1.44	0.63	0.04	0.06	I	2.17	4
1.31	0.80	0.35	0.17			32
1.02	0.52	0.17	0.00			6
2.71	2.54	2.00	1.63	IV	9.06	18
2.60	2.60	2.23	1.80			30
2.69	2.96	2.62	3.12	V	11.39	35

Roselle

A	B	C	D	Subset	GDI	Family
1.71	0.92	0.21	0.25	III	3.32	34
1.56	0.83	0.61	0.00			51
1.80	1.27	0.20	0.25			28
1.69	1.11	0.39	0.47			36
1.90	1.50	0.55	0.71	IV	4.81	13
1.72	1.49	0.82	0.73			30
2.28	1.74	0.46	0.54			7
1.36	0.71	0.07	0.24	II	1.81	21
0.83	0.63	0.30	0.47			47
1.15	0.17	0.00	0.09			4
0.50	0.38	0.38	0.13			55
1.17	0.45	0.06	0.02			6
0.54	0.04	0.00	0.00	I	0.8	32
2.46	2.00	1.00	1.46	V	7.38	18
2.53	2.23	1.15	1.93			35

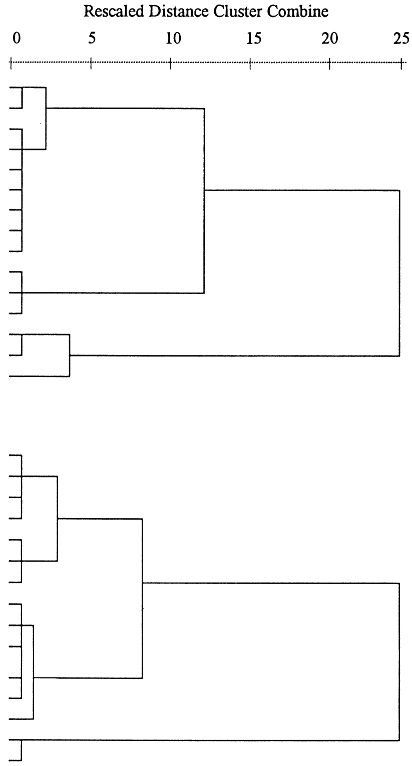

Figure 4. Cannara and Roselle stands. Average damages of each family, cluster composition, subsets (i.e. each homogeneous cluster identified from the less (I) to the most (V) damaged), GDI (sum of the mean of the sum of the four damages of each subset), and dendrogram based on UPGMA cluster analysis from data registered for each family. Distant measures are based on similarity/dissimilarity coefficients.

Table 4. Average damage based on PCA factor scores of the different homogeneous subsets obtained by Cluster Analysis (Figure 4). Means followed by different letters are significantly different according to Scheffe test (α= 0.05)

Cannara			Roselle		
subset	N	harmonic mean	subset	N	harmonic mean
I	3	−0.94 a	I	1	−1.58 a
II	2	−0.12 b	II	5	−1.10 b
III	7	0.56 c	III	4	−0.49 c
IV	2	1.67 d	IV	3	0.08 d
V	1	2.51 e	V	2	1.05 e

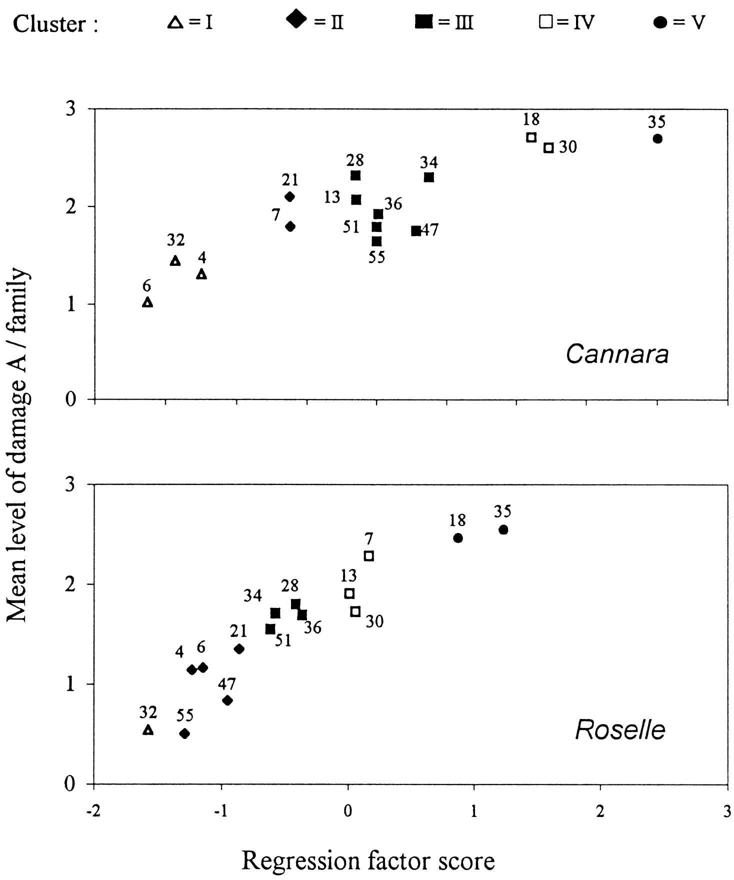

Figure 5. Plot of the mean value of damage A registered for each family against the regression factor scores obtained from the PCA performed on all damages in Cannara and Roselle. Each subset is individuated by a different symbol.

Discussion

In nursery, the families of cypress evaluated already showed clear differences in susceptibility to attack by the eriophyoid mite, allowing a preliminary assignment of the families into a more susceptible and a less susceptible group.

When the seedlings were transplanted in field, some difference in the range of symptoms present was observed in both stands, in comparison with the nursery, *i.e.* damages C and D, absent or sporadic in the nursery, were widely registered, while the intensity of A and B slightly decreased. However the consistency of the allocation of families to susceptibility groups between the nursery and field data showed that on the whole the results from the nursery could be used to provide an indication of the likely susceptibility of plant in the field.

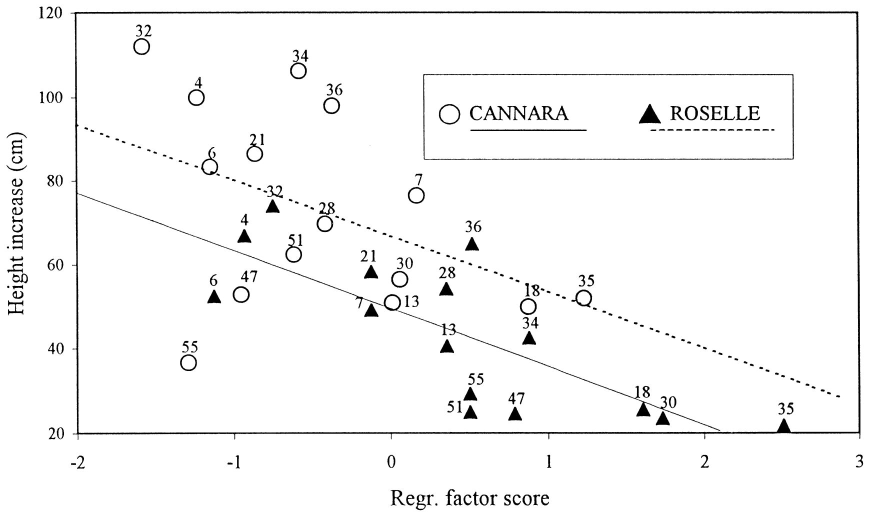

Figure 6. Dispersion plot and regression in Cannara ($R^2 = 0.60$) and in Roselle ($R^2 = 0.21$) of the height increase registered for each family on the regression factor scores obtained from the PCA performed on all damages

With regards to injury pattern, although more than one damage can coexist in the same plant, the following chronology of the symptoms is likely (Castagnoli and Simoni 1998): at first the buds appear enlarged and deformed (damage A) and then partially dried (B); in fact these injuries are usually the most common and at high levels in the nursery and in the first period after transplant. Then, associated with plant growth, damage B can evolve either to C when large areas of the plant whither and die, or to D when the plant reacts by inducing the proliferation of new buds. While damage D is surely ascribable to activity of the eriophyoids, the role played by these mites to determine C must be studied further and also whether other biotic and/or abiotic factors are involved. Since in our five-year observations, the general trend, especially in the most susceptible families, was a considerable increase of those damages (C and D) mainly responsible in the loss of aesthetic value of cypress trees, we can conclude that the plants recover only with difficulty even to partial health. When recovery occurs, it seems mainly due to environmental conditions. In fact, the two considered cypress stands evidenced different injury intensity, although the susceptibility to eriophyoid mites was graduated in an analogous manner in the families. For instance, in Roselle stand the first two groups did not overcome a GDI of 1.81 with only damage A sometimes slightly higher than 1, whereas in Cannara already the first group reached a GDI of 2.17. Furthermore the high susceptibility was always coupled with a very slow growth rate.

The families which differed in their responses between locations, such as families 30, 34, 35, 47, 55 which showed differences in the GDI $\geqslant$ 3 between stands, are unlikely to be widely adaptable in the field. Different localities may imply differences in soil and climatic conditions; in our case the higher humidity registered in Cannara could contribute to the increase of mite population, like in other eriophyoid species (Jeppson et al. 1975; Sabelis and Bruin 1996).

The significant correlation among the four damage categories and between damage categories and plant height increment was important from a practical point of view. In particular, monitoring the intensity of damage category A, which is the most common and easy to diagnose, can give an important indication of the degree of susceptibility of plants in a very early phase. However, studies on the relationship between the injuries described by the damage categories and actual *Trisetacus* infestation, which may help us to better understand the behaviour of the mites in different cypress individuals, are still lacking.

Concerning the canker susceptibility, preliminary observations carried out at Roselle eighteen months after the *Seiridium* inoculation on the sampled plants (Raddi and Panconesi, personal communication) indicated that mite and fungus susceptibility did not always coincide. In the scale of susceptibility to the fungus (level 1 = canker recovery; level 3 = canker with highest growth), our families ranged from 1.97 to 3; usually a level < 2 is considered an index of low susceptibility. In this context, family 4 could be of particular interest for its low susceptibility both to canker and eriophyoids, whereas family 32 (the least damaged by eriophyoid) was, together with family 51, the most susceptible to canker (level 3).

From a practical viewpoint, the evaluation of susceptibility on the basis of injury pattern may assist the selection and certification of families of low susceptibility to eriophyoid mite and, possibly, also to canker.

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